

### **REMARKS/ARGUMENTS**

**Claim status.** Claims 2 to 13 and 25 are pending in this application. Claim 2 is amended hereby. No claim has been added.

**Amendments to the Specification.** The amendments to the specification do not add new matter for the following reasons.

The Applicants substituted "integrin/adhesion" with "adhesion" to accommodate the Examiner, as described hereinbelow. The term "integrin/adhesion antagonist" is defined explicitly in the specification. The Applicants consistently made the aforementioned substitution, including the definition of the term, such that no new disclosure in or removal of disclosure is implied by the amendment. Specifically, the Applicants note that peptides that inhibit integrins or integrin receptors remain within the definition of "adhesion antagonist" and still fall within the claims.

The sequence identified as SEQ ID NO: 10 was erroneously duplicative of SEQ ID NO: 9 and removed by amendment for that reason.

The Applicants added the text describing the X number substituents in response to the Examiner's objection. The specification clearly described the text by reference to the X number substituents, and the added text is the same as in the incorporated documents.

The Applicants attorney has also executed a declaration as mentioned by the Examiner. The Applicants explicitly do not concede that the subject matter added by this amendment constituted essential material under MPEP Section 608.01(p), as the X number substituents do not appear in the claims and are not alleged to be constitute the best mode. Furthermore, the Applicants note that the PCT applications cited correspond to issued U.S. patents, as noted in the foregoing amendment, so that no amendment was necessarily required.

The amendments to the Sequence Listing merely delete SEQ ID NO: 10 and conform the text of the features of SEQ ID NOS: 13-16 to the specification.

**Rejection under Section 112, first paragraph.** The Examiner rejected Claims 2-5, 7-9, 13, and 25 under Section 112, first paragraph.

In making this rejection, the Examiner stated that "peptide, YIGSR, when fused to Fc was proteolyzed" (Office Action at page 4). The Examiner then apparently alleges that only those peptides designed to avoid degradation (SEQ ID NOS: 95 and 96) are enabled (Office Action at page 5).

The specification discusses proteolysis in Example 3, page 57. Example 3 actually states, "Since some proteolysis was seen in laminin-5, the IC100 of laminin-5 could not be assessed accurately." (Specification at page 57, lines 10-11). Laminin-5 is defined at page 57, line 5 as a five-time tandem repeat of the laminin peptide (YIGSR). This statement does not support the Examiner's position in several ways:

- The statement acknowledges "some proteolysis"; the Examiner's position requires proteolysis of every single molecule in a sample, making that sample entirely inactive.
- Despite its acknowledgment of some proteolysis, the statement says that the laminin-5 molecule possessed inhibitory activity. The proteolysis merely caused heterogeneity in the sample such that the IC100 could not be assessed *accurately*. Example 3 still describes laminin-5 as having activity, which is sufficient to satisfy the utility requirement of Section 101 and the enablement requirement of Section 112.
- The statement regarding proteolysis applied only to the laminin-5 molecule. The Examiner's position assumes that proteolysis was seen in all YIGSR peptides when in fact Example 3 does not report proteolysis in the laminin-3 peptide and the laminin-3 Fc fusion, which had IC100's of 2.9  $\mu$ M and 55 nM, respectively (Specification at page 57, lines 7-9).

In short, the specification only reports some proteolysis in laminin-5, and the laminin-5 Fc fusion still has sufficient activity for patentability.

**Rejection under Section 112, second paragraph.** The Examiner rejected Claims 2-5, 7-9, 13, and 25 under Section 112, second paragraph.

Claim 2 was rejected due to use of a slash (/). The Applicants made this amendment to accommodate the Examiner, but hereby expressly do not admit that any indefiniteness is introduced by use of the slash. The slash was part of the term "integrin/ adhesion antagonist," which is explicitly defined at page 5, line 23 to page 6, line 2 and is used consistently throughout the specification. The Applicants thus believe that there is no indefiniteness in the use of the slash in Claim 2.

Claim 13 was rejected for its reference to tables appearing in the specification. Although the Applicants perceive no indefiniteness and hereby explicitly do not admit to any indefiniteness, the Applicants hereby amend Claim 13 to accommodate the Examiner.

**Rejection under Section 103.** The Examiner rejected Claims 2-5, 7-9, 13, and 25 under Section 103 over Whitty *et al.* (US Pat. Appl. 2002/015547) in view of Mu *et al.* (1999), *Biochem. Biophys. Res. Comm.* **255**: 75-9.

Whitty *et al.* describe fusion proteins comprising interferon beta. Whitty *et al.* do not mention laminin or any other adhesion antagonist peptide. Nothing in the Whitty *et al.* reference suggests creating Fc fusions with laminin or any other adhesion antagonist peptide.

Mu *et al.* describe conjugation of laminin peptide to poly(sterene co-maleic acid), referred to as "SMA" in its anhydride form. Mu *et al.* also discuss conjugation of laminin peptide with polyethylene glycol (PEG). Fc, however, differs from PEG and SMA in significant ways. First, Fc is a fragment of a naturally occurring protein and can be produced recombinantly. The Fc-peptide fusion molecule, in turn, can also be produced recombinantly without the chemical processes required for SMA - and PEG-conjugated molecules. Mu *et al.* show no recognition of this advantage in production of Fc-peptide fusion molecules.

Second, Fc is a multimeric molecule (dimeric in IgG Fc, for example) whereas the PEG and SMA employed by Mu *et al.* appear to be monomeric. Nothing in the Mu *et al.* reference suggests that its authors understood this advantage of Fc-peptide fusion.

The Examiner alleged that Mu *et al.* taught that laminin and interferon belong to the family of extracellular matrix domains active in cell adhesion. The Examiner provided no exact citation for this allegation and the Applicants were unable to find any such reference in the Mu *et al.* reference. More important, the comparison between laminin and interferon appears to be inaccurate. Laminin is an adhesion molecule, whereas the interferons are cytokines (see enclosures from Alberts *et al.*, *The Molecular Biology of the Cell*, 2<sup>nd</sup> Ed., Garland Pub. Inc., New York & London, pp. 803, 819, and Kuby, *Immunology*, W.H. Freeman and Company, New York, p. 258. Furthermore, the comparison of interferons to laminin ignores that the application concerns the laminin pentapeptide YIGSR rather than the full 850 kD laminin protein.

For these reasons, the Applicants respectfully submit that there is no suggestion in the art to combine the teachings of Mu *et al.* and Whitty *et al.* and that their teachings, even in combination, fail to encompass the claimed invention.

The Examiner also cited Schachner, U.S. Pat. No. 5,792,743, stating that it suggests a fusion of laminin and Fc (Office Action at page 9). Schachner actually describes Fc fusion with a different molecule (L1--) and the claims are limited as such. Furthermore, Schachner's specification appears to be referring to the full laminin protein, which is a large protein trimer as noted on the enclosure, rather than to the laminin peptide of the present invention. For these reasons, the Applicants respectfully submit that the teachings of Schachner fail to encompass the claimed invention. Furthermore, although the Examiner has not suggested it, the Applicants further submit that there is no suggestion in the art to combine the teachings of Schachner and Whitty *et al.* or Mu *et al.*, and that those teachings fail to encompass the claimed invention even in combination.

**Conclusion.** In view of the foregoing amendments and remarks, the Applicants respectfully request reconsideration of the restriction requirement, entry of all amendments, and allowance of all claims.

Respectfully submitted,



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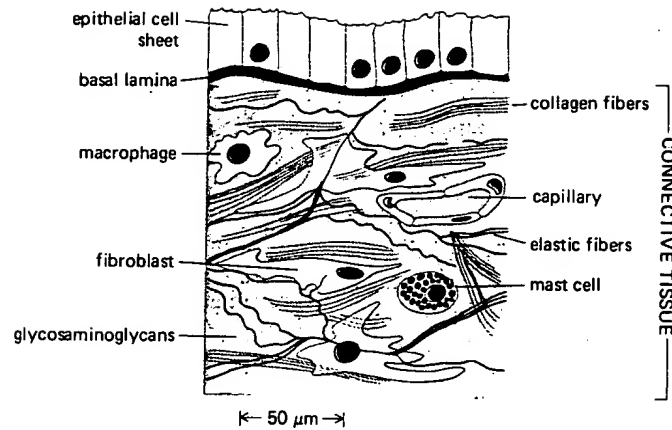


Figure 14-21 The connective tissue underlying an epithelial cell sheet.

part in controlling cell behavior. We shall confine our account to the extracellular matrix of vertebrates, but unique and interesting related structures are seen in many other organisms, such as the cell walls of bacteria and plants, the cuticles of worms and insects, and the shells of mollusks. Plant cell walls are considered in detail in Chapter 20.

Until recently the vertebrate extracellular matrix was thought to serve mainly as a relatively inert scaffolding to stabilize the physical structure of tissues. But now it is clear that the matrix plays a far more active and complex role in regulating the behavior of the cells that contact it—influencing their development, migration, proliferation, shape, and metabolic functions. The extracellular matrix has a correspondingly complex molecular composition; although our understanding of its organization is still fragmentary, there has been rapid progress in characterizing some of its major components.

### The Extracellular Matrix Consists Primarily of Fibrous Proteins Embedded in a Hydrated Polysaccharide Gel

The macromolecules that constitute the extracellular matrix are mainly secreted locally by cells in the matrix. In most connective tissues these macromolecules are secreted largely by *fibroblasts* (Figure 14-22). In some specialized connective tissues, however, such as cartilage and bone, they are secreted by cells of the fibroblast family that have more specific names: chondroblasts, for example, form cartilage, and osteoblasts form bone. The two main classes of extracellular macromolecules that make up the matrix are (1) polysaccharide *glycosaminoglycans* (GAGs), which are usually found covalently linked to protein in the form of *proteoglycans*, and (2) fibrous proteins of two functional types: mainly structural (for example, *collagen* and *elastin*) and mainly adhesive (for example, *fibronectin* and *laminin*). The glycosaminoglycan and proteoglycan molecules form a highly hydrated, gel-like “ground substance” in which the fibrous proteins are embedded. The aqueous phase of the polysaccharide gel permits the diffusion of nutrients, metabolites, and hormones between the blood and the tissue cells; the collagen fibers strengthen and help to organize the matrix, and rubberlike elastin fibers give it resilience. The adhesive proteins help cells attach to the extracellular matrix: fibronectin promotes the attachment of fibroblasts and related cells to the matrix in connective tissues, while laminin promotes the attachment of epithelial cells to the basal lamina.

### Glycosaminoglycan Chains Occupy Large Amounts of Space and Form Hydrated Gels<sup>10</sup>

**Glycosaminoglycans** (GAGs) are long, unbranched polysaccharide chains composed of repeating disaccharide units. They are called glycosaminoglycans because one of the two sugar residues in the repeating disaccharide is always an amino sugar (*N*-acetylglucosamine or *N*-acetylgalactosamine). In most cases this

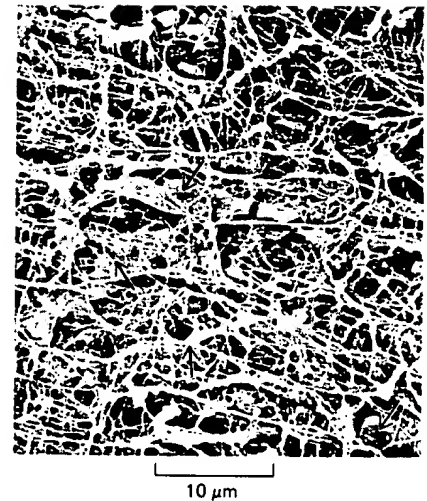


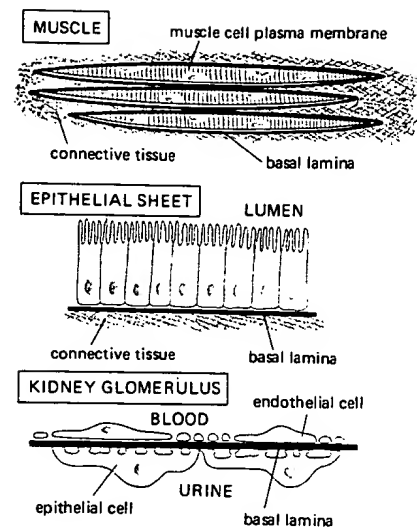
Figure 14-22 Scanning electron micrograph of fibroblasts (arrows) in the connective tissue of the cornea in a chick embryo. The extracellular matrix surrounding the fibroblasts is composed largely of collagen fibers (there are no elastic fibers in the cornea). The glycosaminoglycans, which normally form a hydrated gel filling the interstices of the fibrous network, have collapsed onto the surface of the collagen fibers during the dehydration process necessary for specimen preparation. (Courtesy of Robert Trelstad.)

to form myelin). The basal lamina thus separates these cells and cell sheets from the underlying or surrounding connective tissue. In other locations, such as the kidney glomerulus and lung alveolus, a basal lamina lies between two different cell sheets, where it functions as a highly selective filter (Figure 14–47). However, basal laminae serve more than simple structural and filtering roles. They are able to determine cell polarity, influence cell metabolism, organize the proteins in adjacent plasma membranes, induce cell differentiation, and, like fibronectin, serve as specific “highways” for cell migration.

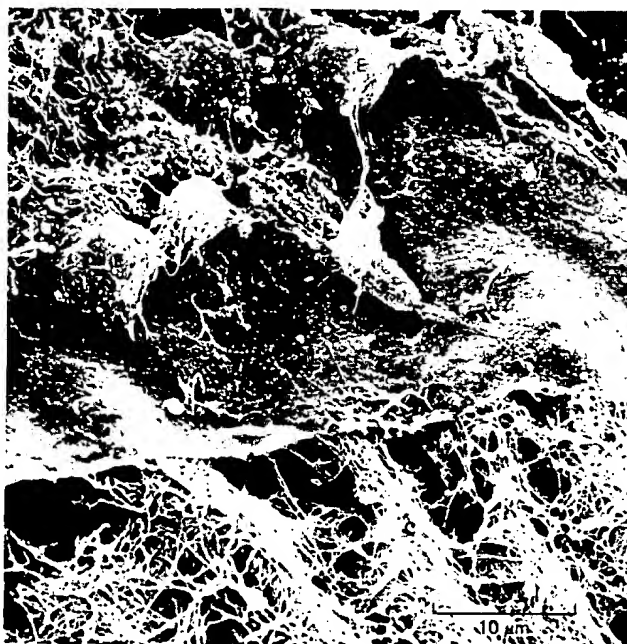
The basal lamina is largely synthesized by the cells that rest on it (Figure 14–48). In essence it is a tough mat of type IV collagen (see Figure 14–42) with specific additional molecules on each face that help bind it to the adjacent cells or matrix. Although the precise composition of basal laminae varies from tissue to tissue and even from region to region in the same lamina (see p. 821), all basal laminae contain type IV collagen together with proteoglycans (primarily heparan sulfates) and the glycoproteins *laminin* and *entactin*. **Laminin** is a large (~850,000 daltons) complex of three very long polypeptide chains arranged in the shape of a cross and held together by disulfide bonds (Figure 14–49). Like fibronectin, it consists of a number of functional domains: one binds to type IV collagen, one to heparan sulfate, and one or more to laminin receptor proteins on the surface of cells. A single dumbbell-shaped entactin molecule is thought to be tightly bound to each laminin molecule where the short arms meet the long one.

As seen in the electron microscope after conventional fixation and staining, most basal laminae consist of two distinct layers: an electron-lucent layer (*lamina lucida* or *rara*) adjacent to the basal plasma membrane of the cells that rest on the lamina—typically epithelial cells—and an electron-dense layer (*lamina densa*) just below. In some cases a third layer containing collagen fibrils (*lamina reticularis*) connects the basal lamina to the underlying connective tissue. Some cell biologists use the term *basement membrane* to describe the composite of all three layers (Figure 14–50), which is usually thick enough to be seen in the light microscope. The detailed molecular organization of the basal lamina is still uncertain, although electron microscopic studies using antibody labeling suggest that the lamina densa is composed primarily of type IV collagen, with proteoglycan molecules located on either side; laminin is thought to be present mainly on the plasma-membrane side of the lamina densa, where it helps to bind epithelial cells to the lamina, while fibronectin helps to bind the matrix macromolecules and connective tissue cells on the opposite side.

The shapes and sizes of some of the major components of the basal lamina and other forms of extracellular matrix are compared in Figure 14–51.



**Figure 14–47** Three ways in which basal laminae (black lines) are organized: surrounding cells (such as muscle cells), underlying epithelial cell sheets, and interposed between two cell sheets (as in the kidney glomerulus). Note that in the kidney glomerulus, both cell sheets have gaps in them, so that the basal lamina serves as the permeability barrier determining which molecules will pass into the urine from the blood. Since the glomerular basal lamina develops as a result of the fusion of two basal laminae, one produced by the endothelial cells and the other by the epithelial cells, it is twice as thick as most basal laminae.



**Figure 14–48** Scanning electron micrograph of a basal lamina in the cornea of a chick embryo. Some of the epithelial cells (E) have been removed to expose the upper surface of the matlike basal lamina (BL). Note the network of collagen fibrils (C) in the underlying connective tissue interacting with the lower face of the lamina. The macromolecules that comprise the basal lamina are synthesized by the epithelial cells that sit on it. (Courtesy of Robert Trelstad.)

antigen or mitogen, IL-4 acts as a growth factor, driving the B cell to replicate its DNA. Finally, in the case of proliferating B lymphocytes, IL-4 acts as a differentiation factor by regulating class switching to the IgG1 and IgE isotypes. In this role IL-4 has been termed a "switch-inducing" factor.

### Interleukin 5 (IL-5)

Interleukin 5, like IL-4, has been shown to stimulate both B-cell proliferation and differentiation. This factor enhances production of IgA; it also appears to act synergistically with IL-4 to enhance production of IgE. Interleukin 5 also induces growth and differentiation of eosinophils.

### Interleukin 6 (IL-6)

Activated  $T_H$  cells, macrophages, monocytes, and fibroblasts and several tumors (e.g., cardiac myxomas, cervical cancer, and bladder cancer) produce IL-6 constitutively. Several myeloma cells have been shown to secrete IL-6, which in this case functions in an autocrine manner to stimulate cell proliferation. Among the other activities of IL-6 is stimulation of immunoglobulin secretion by plasma cells and, in concert with IL-1, it acts as a costimulator of  $T_H$ -cell activation.

### Interleukin 7 (IL-7)

Interleukin 7, first cloned in 1989, induces lymphoid stem cells to differentiate into progenitor B cells. This cytokine was discovered by expressing a cDNA library from bone-marrow stromal cells in Cos cells and testing the supernatants for biological activity. About 720,000 recombinants were screened before a clone possessing IL-7 activity was identified. Since its discovery, IL-7 has been shown to increase expression of IL-2 and IL-2 receptors in resting T cells, thereby inducing T-cell proliferation. In addition, IL-7 stimulates the proliferation of both fetal and adult thymocytes.

### Interleukin 8 (IL-8)

Secreted primarily by monocytes, IL-8 has a variety of effects on neutrophils. For example, in the presence of IL-8 neutrophils adhere to vascular endothelial cells and emigrate from the blood into the tissue compartment toward a concentration gradient of IL-8. This cytokine is such a potent chemotactic factor for neutrophils that nanogram quantities are active.

### Interleukin 9 (IL-9)

A glycoprotein secreted by certain  $T_H$ -cell clones, IL-9 supports the proliferation of  $T_H$  cells in the *absence* of antigen or antigen-presenting cells. It is produced by the  $T_H2$  subset of long-term mouse T-cell clones and may act as an autocrine growth factor during antigen activation. Recently IL-9 has also been shown to promote the growth of mast cells.

### Interleukin 10 (IL-10)

Recently an important regulatory cytokine, *cytokine-synthesis inhibitory factor* (CSIF), or IL-10, has been cloned and characterized. This cytokine is secreted by the  $T_H2$  subset of long-term mouse T-cell clones and suppresses cytokine production by the  $T_H1$  subset. The  $T_H1$  subpopulation secretes IL-2 and IFN- $\gamma$  and has been implicated in macrophage activation in the delayed-type hypersensitivity response. The  $T_H2$  subpopulation secretes IL-4 and IL-5, triggering a predominantly humoral antibody response. The fact that IL-10 secretion by the  $T_H2$  subpopulation suppresses cytokine production by the  $T_H1$  subpopulation confers upon this cytokine a central role in regulating humoral and cell-mediated responses. The  $T_H1$  and  $T_H2$  subsets and the role of IL-10 in some diseases are discussed later in this chapter.

### Interferons (IFNs)

The interferons are a family of glycoproteins, produced by a variety of cell types, that interfere with viral replication and help to regulate the immune response. Interferon alpha (IFN- $\alpha$ ), derived from various leukocytes, and interferon beta (IFN- $\beta$ ), derived from fibroblasts, were the first interferons to be characterized. Interferon gamma (IFN- $\gamma$ ) was discovered later and shown to be secreted by T lymphocytes following antigen or mitogen activation. All three interferons are released from virus-infected cells and confer antiviral protection on neighboring cells. Unlike IFN- $\alpha$  and IFN- $\beta$ , which predominantly function to induce an antiviral state, IFN- $\gamma$  has various pleiotrophic activities including the ability to enhance the functional activity of macrophages,  $T_C$  cells, T cells involved in delayed-type hypersensitivity ( $T_{DTH}$  cells), and NK cells. One of the most interesting effects of IFN- $\gamma$  is the induction of increased class I and class II MHC expression on cells. The increased synthesis of class II MHC molecules by macrophages allows them to function as more effective antigen-presenting cells. Interferon gamma also has antagonistic activity against a number of cytokines. For example, when IFN- $\gamma$  is added together with IL-4 to B cells, the class switch to IgE is blocked.